

Function of a hyaluronidase-like enzyme in the venom of spiders

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In many venoms of mygalomorph and araneomorph spiders a hyaluronidase-like enzyme has been identified which points to its important function as spreading factor. For the venoms of 39 spider species belonging to 21 families the enzymatic activity was analyzed in terms of its hydrolyzing activity towards hyaluronan, chondroitin 4 sulfate, heparin sulfate and dermatan sulfate in an agarose gel electrophoresis. Furthermore, we determined the molecular masses of the enzyme in those venoms with zymograms. The hyaluronidase-like enzyme was purified and characterized from the venom of the Central American spider *Cupiennius salei* and its amino acid sequence was elucidated by cDNA analysis of the venom gland transcriptome. The enzyme structure is characterized by two N-linked glycans and a C-terminal EGF-like domain with a molecular mass of 46.3 kDa as measured by MALDI-TOF-MS. This enzyme exhibited maximal activity at acidic pH in the presence of 0.2 M KCl. The end products of hyaluronan and chondroitin 4 sulfate degradation are tetramers and hexamers, respectively. At the reducing end of hyaluronan and chondroitin 4 sulfate oligomers we have identified terminal N-acetylglucosamine or N-acetylgalactosamine which confirm this enzyme as an endo- β -N-acetyl-D-hexosaminidase hydrolase. Coinjection of recombinant hyaluronidase-like enzyme with CsTx-1, the main neurotoxin in the venom of *C. salei*, into *Drosophila* flies verified the hypothesis of this enzyme acting as spreading factor. Keywords: *Cupiennius salei*, spider, hyaluronidase-like enzyme